

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property
 Organization
 International Bureau



(43) International Publication Date
 10 September 2004 (10.09.2004)

PCT

(10) International Publication Number
WO 2004/076638 A2

(51) International Patent Classification⁷: **C12N**
 (21) International Application Number:
 PCT/US2004/005654
 (22) International Filing Date: 25 February 2004 (25.02.2004)
 (25) Filing Language: English
 (26) Publication Language: English
 (30) Priority Data:
 10/374,780 25 February 2003 (25.02.2003) US
 10/675,852 30 September 2003 (30.09.2003) US

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(63) Related by continuation (CON) or continuation-in-part (CIP) to earlier application:
 US 10/374,780 (CIP)
 Filed on 25 February 2003 (25.02.2003)

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(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Declaration under Rule 4.17:

— of inventorship (Rule 4.17(iv)) for US only

Published:

— without international search report and to be republished upon receipt of that report

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: POLYNUCLEOTIDES AND POLYPEPTIDES IN PLANTS

(57) Abstract: The invention relates to plant transcription factor polypeptides, polynucleotides that encode them, homologs from a variety of plant species, and methods of using the polynucleotides and polypeptides to produce transgenic plants having advantageous properties compared to a reference plant. Sequence information related to these polynucleotides and polypeptides can also be used in bioinformatic search methods is also disclosed.

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sequence shown in the Sequence Listing due to degeneracy in the genetic code, are also within the scope of the invention.

Altered polynucleotide sequences encoding polypeptides include those sequences with deletions, insertions, or substitutions of different nucleotides, resulting in a polynucleotide encoding a polypeptide with at least one functional characteristic of the instant polypeptides. Included within this definition are polymorphisms which may or may not be readily detectable using a particular oligonucleotide probe of the polynucleotide encoding the instant polypeptides, and improper or unexpected hybridization to allelic variants, with a locus other than the normal chromosomal locus for the polynucleotide sequence encoding the instant polypeptides.

Allelic variant refers to any of two or more alternative forms of a gene occupying the same chromosomal locus. Allelic variation arises naturally through mutation, and may result in phenotypic polymorphism within populations. Gene mutations can be silent (i.e., no change in the encoded polypeptide) or may encode polypeptides having altered amino acid sequence. The term allelic variant is also used herein to denote a protein encoded by an allelic variant of a gene. Splice variant refers to alternative forms of RNA transcribed from a gene. Splice variation arises naturally through use of alternative splicing sites within a transcribed RNA molecule, or less commonly between separately transcribed RNA molecules, and may result in several mRNAs transcribed from the same gene. Splice variants may encode polypeptides having altered amino acid sequence. The term splice variant is also used herein to denote a protein encoded by a splice variant of an mRNA transcribed from a gene.

Those skilled in the art would recognize that, for example, G481, SEQ ID NO: 88, represents a single transcription factor; allelic variation and alternative splicing may be expected to occur. Allelic variants of SEQ ID NO: 87 can be cloned by probing cDNA or genomic libraries from different individual organisms according to standard procedures. Allelic variants of the DNA sequence shown in SEQ ID NO: 87, including those containing silent mutations and those in which mutations result in amino acid sequence changes, are within the scope of the present invention, as are proteins which are allelic variants of SEQ ID NO: 88. cDNAs generated from alternatively spliced mRNAs, which retain the properties of the transcription factor are included within the scope of the present invention, as are polypeptides encoded by such cDNAs and mRNAs. Allelic variants and splice variants of these sequences can be cloned by probing cDNA or genomic libraries from different individual organisms or tissues according to standard procedures known in the art (see USPN 6,388,064).

Thus, in addition to the sequences set forth in the Sequence Listing, the invention also encompasses related nucleic acid molecules that include allelic or splice variants of SEQ ID NO: 2N - 1, wherein N = 1- 229, SEQ ID NO: 459-466; 468-487; 491-500; 504; 506-511; 516-520; 523-524; 527; 529; 531-533; 538-539; 541-557; 560-568; 570-586; 595-596; 598-606; 610-620; 627-634; 640-664; 670-707; 714-719; 722-735; 740-741; 743-779; 808-823; 825-834; 838-850; 855-864; 868-889; 892-902; 908-909; 914-921; 924-925; 927-932; 935-942; 944-952; 961-965; 968-986; 989-993; 995-1010; 1012-1034; 1043-1063; 1074-1080; 1091-1104; 1111-1121; 1123-1128; 1134-1138; 1142-1156; 1159-1175;

1187-1190; 1192-1199; 1202-1220; 1249-1253; 1258-1262; 1264-1269; 1271-1287; 1292-1301; 1303-1309; 1315-1323; 1328-1337; 1340-1341; 1344-1361; 1365-1377; 1379-1390; 1393-1394; 1396-1398; 1419-1432; 1434-1452; 1455-1456; 1460-1465; 1468-1491; 1499; 1502; 1505-1521; 1523-1527; 1529-1532; 1536-1539; 1542-1562; 1567-1571; 1573-1582; 1587-1592; 1595-1620; 1625-1644; 1647-1654; 5 1659-1669; 1671-1673; 1675-1680; 1682-1686; 1688-1700; 1706-1709; 1714-1726; 1728-1734; 1738-1742; 1744-1753; 1757-1760; 1763-1764; 1766-1768; 1770-1780; 1782-1784; 1786-1789; 1791-1804; 1806-1812; 1814-1837; 1847-1856; 1858-1862; 1864-1873; 1876-1882; 1885-1896; 1902-1910; 1913-1916; 1921-1928; 1931-1936; 1940-1941; 1944-1946, 2907-2941, 2944, 2945, 2947, 2949, or SEQ ID NO: 2N - 1, wherein N = 974- 1101, and include sequences which are complementary to any of the 10 above nucleotide sequences. Related nucleic acid molecules also include nucleotide sequences encoding a polypeptide comprising or consisting essentially of a substitution, modification, addition and/or deletion of one or more amino acid residues compared to the polypeptide as set forth in any of SEQ ID NO: 2N, wherein N = 1- 229, SEQ ID NO: 467; 488-490; 501-503; 505; 512-515; 521-522; 525-526; 528; 530; 534-537; 540; 558-559; 569; 587-594; 597; 607-609; 621-626; 635-639; 665-669; 708-713; 15 720-721; 736-739; 742; 780-807; 824; 835-837; 851-854; 865-867; 890-891; 903-907; 910-913; 922-923; 926; 933-934; 943; 953-960; 966-967; 987-988; 994; 1011; 1035-1042; 1064-1073; 1081-1090; 1105-1110; 1122; 1129-1133; 1139-1141; 1157-1158; 1176-1186; 1191; 1200-1201; 1221-1248; 1254-1257; 1263; 1270; 1288-1291; 1302; 1310-1314; 1324-1327; 1338-1339; 1342-1343; 1362-1364; 1378; 1391-1392; 1395; 1399-1418; 1433; 1453-1454; 1457-1459; 1466-1467; 1492-1498; 1500-1501; 1503-20 1504; 1522; 1528; 1533-1535; 1540-1541; 1563-1566; 1572; 1583-1586; 1593-1594; 1621-1624; 1645-1646; 1655-1658; 1670; 1674; 1681; 1687; 1701-1705; 1710-1713; 1727; 1735-1737; 1743; 1754-1756; 1761-1762; 1765; 1769; 1781; 1785; 1790; 1805; 1813; 1838-1846; 1857; 1863; 1874-1875; 1883-1884; 1897-1901; 1911-1912; 1917-1920; 1929-1930; 1937-1939; 1942-1943; 2942 or 2943, 2945, 2947, 2949, or SEQ ID NO: 2N, wherein N = 974- 1101. Such related polypeptides may comprise, for example, 25 additions and/or deletions of one or more N-linked or O-linked glycosylation sites, or an addition and/or a deletion of one or more cysteine residues.

For example, Table 4 illustrates, e.g., that the codons AGC, AGT, TCA, TCC, TCG, and TCT all encode the same amino acid: serine. Accordingly, at each position in the sequence where there is a codon encoding serine, any of the above trinucleotide sequences can be used without altering the encoded 30 polypeptide.

251	G1266	OE	Increased tolerance to disease Growth regulation; nutrient uptake	Increased tolerance to <i>Erysiphe</i> Altered C/N sensing
253	G1275	OE	Altered architecture	Reduced apical dominance
255	G1305	OE	Increased tolerance to abiotic stress	Reduced chlorosis in heat
257	G1322	OE	Increased tolerance to abiotic stress Altered size Leaf glucosinolates Altered light response and/or shade tolerance Growth regulation; nutrient uptake	Increased seedling vigor in cold Reduced size Increase in M39480 Constitutive photomorphogenesis Altered C/N sensing: increased tolerance to low nitrogen
259	G1323	OE	Altered seed oil Altered seed protein	Decreased seed oil Increased seed protein
261	G1330	OE	Altered hormone sensitivity	Ethylene insensitive when germinated in the dark on ACC
263	G1331	OE	Altered light response and/or shade tolerance Growth regulation; nutrient uptake	Constitutive photomorphogenesis Altered C/N sensing
265	G1332	OE	Altered trichomes Growth regulation; nutrient uptake	Reduced trichome density Altered C/N sensing
267	G1363	OE	Increased tolerance to disease	Increased tolerance to <i>Fusarium</i>
269	G1411	OE	Altered architecture	Loss of apical dominance
2607	G1412	KO	Altered light response and/or shade tolerance	Increased shade tolerance; lack of shade avoidance phenotype
271	G1417	KO	Altered seed oil	Increase in 18:2, decrease in 18:3 fatty acids
273	G1419	OE	Altered seed protein	Increased seed protein
275	G1449	OE	Altered flower	Altered flower structure
277	G1451	OE OE KO	Altered size Altered leaf Altered seed oil	Increased plant size Large leaf size Altered seed oil content
279	G1452	OE	Altered trichomes Altered leaf Altered hormone sensitivity Altered flowering time Increased tolerance to abiotic and osmotic stress	Reduced trichome density Altered leaf shape, dark green color Reduced sensitivity to ABA Better germination on sucrose, salt Late flowering Increased tolerance to drought
281	G1463	OE	Altered senescence	Premature senescence
283	G1471	OE	Altered seed oil	Increased seed oil content
285	G1478	OE	Altered seed protein Altered flowering time Altered seed oil	Decreased seed protein content Late flowering Increased seed oil content
287	G1482	KO OE	Altered pigment Altered root	Increased anthocyanins Increased root growth
289	G1488	OE	Altered seed protein Altered light response and/or shade tolerance Altered architecture	Altered seed protein content Constitutive photomorphogenesis Reduced apical dominance, shorter stems
291	G1494	OE	Altered flowering time	Early flowering

			indeterminate growth
	Altered shoot meristem development Stem bifurcations: G390; G391		Ornamental modification of plant architecture, manipulation of growth and development, increase in leaf numbers, modulation of branching patterns to provide improved yield or biomass
	Altered branching pattern	G427; G568; G988; G1543; G1794	Ornamental modification of plant architecture, improved lodging resistance
	Apical dominance Reduced apical dominance: G47; G211; G1255; G1275; G1411; G1488; G1794; G2509		Ornamental modification of plant architecture
	Altered trichome density; development, or structure Reduced or no trichomes: G225; G226; G247; G585; G676; G682; G1332; G1452; G1816 Ectopic trichomes/altered trichome development/cell fate: G247; G362; G370; G676; G2826 Increase in trichome number, size or density: G362; G634; G838; G2838		Ornamental modification of plant architecture, increased plant product (e.g., diterpenes, cotton) productivity, insect and herbivore resistance
	Stem morphology and altered vascular tissue structure	G47; G438; G748; G988; G1488	Modulation of lignin content; improvement of wood, palatability of fruits and vegetables
	Root development Increased root growth and proliferation: G1482 Increased root hairs: G225; G226; G1816		Improved yield, stress tolerance; anchorage
	Altered seed development, ripening and germination	G979	
	Cell differentiation and cell proliferation	G1540	Increase in carpel or fruit development; improve regeneration

G779, G988, G1075, G1140, G1499, G1947, G2143, G2557 and their functional equivalents, possess reduced fertility; flowers are infertile and fail to yield seed. These could be desirable traits, as low fertility could be exploited to prevent or minimize the escape of the pollen of genetically modified organisms (GMOs) into the environment.

5 The alterations in shoot architecture seen in the lines transformed with G47, G1063, G1645, G2143, and their functional equivalents indicates that these genes and their equivalents can be used to manipulate inflorescence branching patterns. This could influence yield and offer the potential for more effective harvesting techniques. For example, a "self pruning" mutation of tomato results in a determinate growth pattern and facilitates mechanical harvesting (Pnueli et al. (2001) *Plant Cell* 13(12): 2687-702).

10 One interesting application for manipulation of flower structure, for example, by introduced transcription factors could be in the increased production of edible flowers or flower parts, including saffron, which is derived from the stigmas of *Crocus sativus*.

 Genes that later silique conformation in brassicates may be used to modify fruit ripening processes in brassicates and other plants, which may positively affect seed or fruit quality.

15 A number of the presently disclosed transcription factors may affect the timing of phase changes in plants. Since the timing or phase changes generally affects a plant's eventual size, these genes may prove beneficial by providing means for improving yield and biomass.

General development and morphology: shoot meristem and branching patterns. Several of the
20 presently disclosed transcription factor genes, including G390 and G391, and G1794, when introduced into plants, have been shown to cause stem bifurcations in developing shoots in which the shoot meristems split to form two or three separate shoots. These transcription factors and their functional equivalents may thus be used to manipulate branching. This would provide a unique appearance, which may be desirable in ornamental applications, and may be used to modify lateral branching for use in the
25 forestry industry. A reduction in the formation of lateral branches could reduce knot formation. Conversely, increasing the number of lateral branches could provide utility when a plant is used as a view- or windscreen.

General development and morphology: apical dominance: The modified expression of presently
30 disclosed transcription factors (e.g., G47, G211, G1255, G1275, G1411, G1488, G1794, G2509 and their equivalents) that reduce apical dominance could be used in ornamental horticulture, for example, to modify plant architecture, for example, to produce a shorter, more bushy stature than wild type. The latter form would have ornamental utility as well as provide increased resistance to lodging.

35 General development and morphology: trichome density, development or structure. Several of the presently disclosed transcription factor genes have been used to modify trichome number, density, trichome cell fate, amount of trichome products produced by plants, or produce ectopic trichome

SEQUENCE LISTING

<110> Sherman, Bradley K
 Riechmann, Jose Luis
 Jiang, Cai-Zhong
 Heard, Jacqueline E
 Haake, Volker
 Creelman, Robert A
 Ratcliffe, Oliver
 Adam, Luc J
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 Keddie, James
 Dubell III, Arnold N
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<120> POLYNUCLEOTIDES AND POLYPEPTIDES IN PLANTS

<130> MBI-0047 PCT

<150> 10/374,780

<151> 2003-02-25

<150> 10/675,852

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Tyr Gln Thr Ser Asp Val Ala Gly Ala Leu Phe Ser Gly Ser Ser Ser
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Cys Phe Ser His Pro Glu Ser Pro Ser Thr Lys Thr Tyr Val Ala Ala
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aatcgagagg acgccagggc cgattctctg cgtgacttgg ggcgtcgtcg ccgagttttc	180
tccggccgag ggcggcgca ccggacaacc agcgtgagcg cgaccggcga catgactcgc	240
ggcaaccagc gtgaccgca cctgagcgc gcggcggcgc ggaagcccaa cgccaagaac	300

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tcccaggacg ggctcacccc ggagcagcgc cgcgagaggg acaagaaagc tctggaggag	360
aaggcggcca agaaggcgca gcaggcagcg gccggcggca ccgggacctc cacggacaac	420
aacaagaaca aggcaggtgg caagaagtag gaggcagtgg cgcgccgcca cctctgtacg	480
attaatcgat gccttgagct tgtaacttgt tcgcatcctt gtaggtgcga tgcttggtgg	540
gtcataaact ctgatgatat cgagatttgg tgatcagaaa taatgcgtgg ttttcgcgcc	600
caaaattggg aaccccagg ccggccgggg ttattcaggc aggcgcattg actgatacac	660
atatatacaa caagcaaggc ggctagctac agcggctagt gcgcgcgtgg tgcaggcgca	720
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gataaaatac tctagaagct atcgctgtag ccggattaaa ttctgacctt cttgtatagt	1560
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<210> 1532

<211> 999

<212> DNA

<213> Zea mays

<220>

<223> Predicted polypeptide sequence is orthologous to G1275

<400> 1532

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gttccaacac attctagcta cgtgacctcg acgtgcgtgt aataactgat ggatgacgga	180
tcggatcagt acgacgactc cgaggaatga gcaaccgccg ccgcggcgctg cagctgcgcc	240
tcccaccacg cgtcacaggc ggcgctccag ctgggcgctg cagccagagg cgctggcgcc	300

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 gaggaggcgg gcgcgggagg cgggtagtag gcggcgtagc tcgtctcgtg ggcgagtcct 960
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 <211> 194
 <212> PRT
 <213> Oryza sativa

<220>
 <223> Orthologous to G1275

<400> 1533

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Ser Tyr Ser Tyr Ser Ser Ser Pro Phe Met Ala Ser Tyr Thr Pro Glu
 20 25 30

Phe Ser Ala Ala Ala Ile Asp Ala Asn Leu Phe Ser Gly Glu Leu Asp
 35 40 45

Phe Asp Cys Ser Leu Pro Ala Pro Ala Gln Glu Tyr Pro Glu Asn Glu
 50 55 60

Asn Thr Met Met Arg Tyr Glu Ser Glu Glu Lys Met Arg Ala Arg Val
 65 70 75 80

Asn Gly Arg Ile Gly Phe Arg Thr Arg Ser Glu Val Glu Ile Leu Asp
 85 90 95

Asp Gly Phe Lys Trp Arg Lys Tyr Gly Lys Lys Ala Val Lys Asn Ser
 100 105 110

Pro Asn Pro Arg Asn Tyr Tyr Arg Cys Ser Thr Glu Gly Cys Asn Val

115

120

125

Lys Lys Arg Val Glu Arg Asp Arg Glu Asp His Arg Tyr Val Ile Thr
130 135 140

Thr Tyr Asp Gly Val His Asn His Ala Ser Pro Ala Ala Ala Ala Ala
145 150 155 160

Ala Leu Gln Tyr Ala Ala Ala Ala Gly Asp Tyr Tyr Ser Pro Pro Leu
165 170 175

Ser Ser Ala Gly Ser Pro Pro Ala Ala Tyr Ser Ala Gly Gly Ser Leu
180 185 190

Leu Phe

<210> 1534

<211> 246

<212> PRT

<213> Oryza sativa

<220>

<223> Orthologous to G1275

<400> 1534

Met Ser Ser Leu Tyr Pro Ser Leu Leu Ser Leu Ser Glu Ser Pro Ala
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Glu Tyr Arg Gln Val Gly Gly Gly Arg Tyr Ala Gly Glu Asp Val Val
20 25 30

Asp Asp Asp Asp Asp Met Ala Ala Val Ala Asp Ala Val Ser Ser Tyr
35 40 45

Leu Ser Phe Asp Met Asp Asp Val Glu Tyr Tyr Thr Pro Glu Val Gly
50 55 60

Phe His Ser Lys Gln His Asn Pro Pro Pro Val Ala Ala Ala Pro Leu
65 70 75 80

Glu Ala Gly Gly Gly Arg Glu Gln Ser Arg Arg Glu Ala Ala Val Asn
85 90 95

Leu Gly Lys Met Asp Arg Gly Pro Ala Pro Val Ser Gly Gly Ala Ala
100 105 110

Thr Gly Gly Val Pro Arg Ser Lys Asn Gly Ser Lys Ile Ala Phe Lys
115 120 125

Thr Arg Ser Glu Val Asp Val Leu Asp Asp Gly Tyr Arg Trp Arg Lys
130 135 140

Tyr Gly Lys Lys Met Val Lys Asn Ser Pro Asn Pro Arg Asn Tyr Tyr
145 150 155 160

Arg Cys Ser Ser Glu Gly Cys Arg Val Lys Lys Arg Val Glu Arg Ala
165 170 175

Arg Asp Asp Ala Arg Phe Val Val Thr Thr Tyr Asp Gly Val His Asn
180 185 190

His Pro Ala Pro Leu His Leu Arg Pro Gln Leu Pro Pro Pro Gly Gly
195 200 205

Tyr Ser Ile Ala Gly Ala Pro Ala Val Val Ala Pro His Gly Arg Leu
210 215 220

Gly Leu Glu Glu Ala Glu Val Ile Ala Leu Phe Arg Gly Thr Thr Ala
225 230 235 240

Thr Ser Leu Leu Leu Pro
245

<210> 1535
<211> 219
<212> PRT
<213> Oryza sativa

<220>
<223> orthologous to G1275

<400> 1535

Met Ala Ala Ser Leu Gly Leu Cys His Glu Thr Ser Tyr Ala Tyr Ser
1 5 10 15

Tyr Pro Ala Ser Asn Thr Ser Ser Ser Leu Cys Phe Pro Pro Leu Met
20 25 30

Ala Asp His Ile Val Asp Gly Gly Gly Gly Gly Gly Cys Ser Phe Gly
35 40 45

Glu Phe Leu Glu Leu Gly His Ser Val Tyr Ser Leu Pro Leu Pro Pro
50 55 60

Pro Pro Ser Gln Pro Val Val Val Ala Gly Gly Asn Asn Asp Gln Tyr
65 70 75 80

Gly Val Ser Ser Ser Ser Ser Ala Ala Ala Thr Thr Ser Arg Ile Gly
85 90 95

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Phe Arg Thr Arg Ser Glu Val Glu Val Leu Asp Asp Gly Phe Lys Trp
100 105 110

Arg Lys Tyr Gly Lys Lys Ala Val Lys Ser Ser Pro Asn Pro Arg Asn
115 120 125

Tyr Tyr Arg Cys Ser Ala Ala Gly Cys Gly Val Lys Lys Arg Val Glu
130 135 140

Arg Asp Gly Asp Asp Pro Arg Tyr Val Val Thr Thr Tyr Asp Gly Val
145 150 155 160

His Asn His Ala Thr Pro Gly Cys Val Gly Gly Gly Gly His Leu Pro
165 170 175

Tyr Pro Thr Ser Ala Ala Pro Pro Trp Ser Val Pro Ala Ala Ala
180 185 190

Ser Pro Pro Pro Ala His Ala Gln Ala Trp Gly Ala Pro Leu His Ala
195 200 205

Ala Ala Ala Ala His Ser Ser Glu Ser Ser Phe
210 215